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# Mechanisms of Hydrogen Peroxide Decomposition in Soils

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The rates and mechanisms of hydrogen peroxide ($H_2O_2$) decomposition were examined in a series of soil suspensions at $H_2O_2$ concentrations comparable to those found in rainwaters. The formation of hydroxyl radical (OH) as a possible decomposition intermediate was investigated using a new, highly sensitive method. In surface soils with higher organic matter or manganese content, $H_2O_2$ usually decayed rapidly, with disproportionation to water and dioxygen dominating the decomposition, whereas the formation of the hydroxyl radical (OH) represented <10% of the total $H_2O_2$ decomposed. In contrast, for soils with lower organic matter content, $H_2O_2$ usually decayed much more slowly, but OH was a major product of the $H_2O_2$ decomposed. The decomposition was principally associated with soil particles, not the soil supernatant. Different sterilization techniques indicated that decomposition of $H_2O_2$ was at least partly due to biological activity. Because the loss of $H_2O_2$ can largely be accommodated by the production of $O_2$ and OH within these soils, our results suggest that disproportionation through a catalase-type mechanism and the production of OH through a Haber-Weiss mechanism represent the principal routes through which $H_2O_2$ is lost.

## Introduction

A substantial amount of hydrogen peroxide ($H_2O_2$) can be introduced to soils through wet and dry atmospheric deposition. For example, rainwaters are known to contain $H_2O_2$ at concentrations ranging from < 2 $\mu$M to > 40 $\mu$M (*1*). This large amount of deposited oxidant could have a significant impact on soil chemistry especially if its decomposition within the soil leads to the formation of hydroxyl radical (OH) through Fenton-type chemistry (*2*)

$$H_2O_2 + M^{n+} \rightarrow OH + OH^- + M^{n+1} \quad (1)$$

where $M^{n+}$ and $M^{n+1}$ represent the reduced and oxidized form, respectively, of a redox-active metal. The hydroxyl radical is an extremely powerful oxidant, which reacts with most organic and many inorganic compounds at rates at or near the diffusion limit (*3*). Oxidative processes initiated by OH could substantially alter the nature and speciation of the organic and inorganic constituents within the soil.

Millimolar quantities of $H_2O_2$ have also been employed to remediate contaminated soils and sediments. The addition of $H_2O_2$ is thought to produce OH through Fenton-type chemistry and thus to oxidize contaminating species. However, although $H_2O_2$ is known to decompose in these systems, much less is known about the mechanisms of this decomposition in natural and remediated soils (*4*−*6*). More importantly, very little is known about what fraction of the decomposed $H_2O_2$ leads to OH formation and what factors affect the efficiency of this formation (*7*).

The decomposition of $H_2O_2$ in soils may proceed through a number of mechanisms, the first of which can be most simply described by reactions 2−5:

$$-Fe^{3+} + H_2O_2 \rightarrow -Fe^{2+} + O_2^- + 2H^+ \quad (2)$$

$$O_2^- + -Fe^{3+} \rightarrow -Fe^{2+} + O_2 \quad (3)$$

$$O_2^- + -Fe^{2+} + 2H^+ \rightarrow -Fe^{3+} + H_2O_2 \quad (4)$$

$$-Fe^{2+} + H_2O_2 \rightarrow -Fe^{3+} + OH + OH^- \quad (5)$$

Here, $-Fe^{3+}$ represents iron in a liganded form or occupying a site at an oxide surface.[8−10] The sum of reactions 2−5 represents the Fe-catalyzed Haber-Weiss reaction

$$O_2^- + H_2O_2 \rightarrow O_2 + OH + OH^- \quad (6)$$

If the OH generated in reaction 6 reacts exclusively with $H_2O_2$

$$OH + H_2O_2 \rightarrow H_2O + O_2^- + H^+ \quad (7)$$

the net reaction becomes

$$2H_2O_2 \rightarrow O_2 + 2H_2O \quad (8)$$

Reaction 8 represents an Fe-catalyzed disproportionation reaction proceeding through odd-electron (radical) intermediates. This mechanism will produce OH in high yield during $H_2O_2$ decomposition, with the precise yield depending on the relative amounts of existing, redox-active ferrous iron and whether the iron acts catalytically or stoichiometrically. At sufficiently high concentrations, organic and inorganic soil constituents will react with OH in place of the $H_2O_2$, thus decreasing the rate of $H_2O_2$ decomposition and lowering the yield of evolved $O_2$. Under these conditions, the iron acts to catalyze the oxidation of reduced inorganic and organic compounds by $H_2O_2$ via OH. Other metals may catalyze this set of reactions as well.

Disproportionation (reaction 8) can also take place through an even or two-electron process as is achieved by the enzyme catalase, which is found ubiquitously within aerobic organisms. In this case, the hydroxyl radical is not produced during the decomposition of $H_2O_2$ to $O_2$ and $H_2O$, and soil constituents are not subject to oxidation by $H_2O_2$. Manganese oxides and some manganese complexes may be capable of performing this type of disproportionation as well (*4, 11, 12*).

Hydrogen peroxide can also be decomposed by peroxidic-type reactions

$$S_{RED} + H_2O_2 \rightarrow S_{OX} + H_2O \quad (9)$$

where $H_2O_2$ oxidizes a reduced soil constituent ($S_{RED}$) via a two-electron process while being itself reduced to $H_2O$. This

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**TABLE 1. Chemical Composition of Soils**

| soil type and horizon | depth, cm | pH | OM, %[a] | CEC[b] mequiv/100 g | particle size analysis, % sand | silt | clay | Mn, ppm | Fe,[c] g/kg | form of iron oxide |
|---|---|---|---|---|---|---|---|---|---|---|
| Marsh A | | 3−4 | 2.41 | | | | | | | |
| Elms C | | | 1.16 | | | | | | | |
| Peat A | | 3−4 | 65.54 | | | | | | | |
| Galestown Ap[d] | 0−25 | 5.3 | 0.32 | 1.7 | 77.0 | 19.0 | 4.0 | 99 | 0.3 | |
| Bertie Ap[d] | 0−22 | 5.4 | 2.33 | 8.3 | 28.0 | 61.0 | 11.0 | 286 | 0.65 | |
| Berryland E[e] | | 3.8 | 0.08 | 2.0 | | | | | 0.56 ± 0.04 | goethite |
| Berryland BHS[e] | | 3.7 | 2.3 | 41 | | | | | 0.83 ± 0.07 | goethite |
| Jackland A[f,g] | 0−11 | 4.8 | 3.4 | 28 | 24.5 | 60.2 | 15.3 | high Mn nodules | 25.5 ± 1.1 | goethite |
| Myersville Ap[f,g] | 0−8 | 4.5 | 3.8 | 20 | 20.6 | 60.0 | 19.4 | 1970 | 33.3 ± 1.9 | goethite |
| Christiana Ap[f,g] | 0−22 | 4.6 | 7.9 | 21 | 48.8 | 37.6 | 13.6 | 125 | 15.6 ± 0.4 | goethite |
| Christiana Bt[f,g] | 9−18 | 4.3 | 1.1 | 13 | 12.7 | 57.3 | 30.0 | 63 | 22.1 ± 0.9 | hematite |

[a] Percentage of organic matter was obtained from the soil testing lab at the University of Maryland using the loss of ignition technique. [b] Exchangeable cations. [c] Total iron concentrations of the soils were found by atomic absorption using a total digestion method (*23*). [d] Reference (*20*). [e] Reference (*21*). [f] Reference (*19*). [g] Reference (*22*).

type of reaction is catalyzed by the peroxidase enzymes; whether this reaction can be catalyzed by abiotic metal species within the soils is, to our knowledge, unknown.

Along with the stoichiometric oxidation of $H_2O_2$ to $O_2$, these three types of $H_2O_2$ decomposition mechanisms, namely the Haber-Weiss, the catalase-type, and the peroxidase-type, encompass the spectrum of $H_2O_2$ decay pathways. The oxidation of $H_2O_2$ leads to the stoichiometric formation of $O_2$ and no OH, whereas decomposition of $H_2O_2$ by a catalase-type mechanism produces $H_2O$ and $O_2$ but no OH (reaction 8); a peroxidase-type mechanism produces only oxidized substrate and $H_2O$ but no $O_2$ or OH. In contrast, the products of the metal-catalyzed Haber-Weiss reaction will fall between these limiting cases, potentially producing both $O_2$ and OH depending on the relative concentrations of $H_2O_2$ and OH-reactive soil constituents. However, at the relatively low concentrations of $H_2O_2$ employed in our experiments, the oxidation of soil constituents by OH should dominate its loss and thus little $O_2$ production will be obtained.

Here we examine the kinetics of $H_2O_2$ decomposition and its relationship to $O_2$ and OH production in a number of natural soil suspensions using concentrations of $H_2O_2$ comparable to that found in rainwaters. These data are then employed to infer the major pathways of $H_2O_2$ decomposition (see above). A new technique is employed to quantify OH production in these samples. This technique uses the rapid reaction of dimethyl sulfoxide (DMSO) with OH to generate a methyl radical, which is then trapped with 3-amino-2,2,5,5-tetramethyl-1-pyrrolidinyloxy (AP) to form a stable *O*-methylhydroxylamine. Following derivatization with fluorescamine, the product is separated by HPLC and quantified fluorometrically (*13*−*18*).

## Experimental Section

**Chemicals.** Catalase (308 000 units/ml), horseradish peroxidase (HRP) (1310 units/mg, Lot 26H9512), crystalline p-hydroxyphenyl acetic acid (POHPA), tris hydroxymethyl aminomethane (Tris), and fluorescamine were obtained from Sigma Chemical Company. Boric acid (99.99%), sodium phosphate, sodium hydroxide (99.99%), dimethyl sulfoxide (99.99%), 3- amino-2,2,5,5- tetramethyl-1-pyrrolidinyloxy (AP), ferrous sulfate, sodium azide, and formaldehyde were obtained from Aldrich. Hydrogen peroxide 30% (w/w) was obtained from J. T. Baker. HPLC grade methanol, acetonitrile, and glacial acetic acid were obtained from Fisher. Ethanol was purchased from Pharmcoproducts Inc. All chemicals were used as received.

Fluorescamine (3.6 mM) and AP solutions were prepared daily in acetonitrile and water, respectively, and stored in the dark at room temperature. Phosphate buffer (0.1 M, pH = 7.4) and borate buffer (1 M, pH = 8.4) were prepared with distilled, deionized (Milli-Q) water.

A series of soils were obtained from Dr. Bruce James, Department of Agronomy, University of Maryland, and stored at 4 °C. The soil samples were sieved through a 2 mm linear polyethylene sieve, homogenized by mixing, and stored field moist. Selected chemical data and soil characteristics are summarized in Table 1.

**Apparatus.** Hydrogen peroxide measurements employed a SLM AB-2 spectrofluorometer with excitation at 313 nm and emission at 400 nm (4 nm band-passes in each case). The HPLC system, employed for the analysis of OH, has been described previously (*13*−*18*). Separations were performed isocratically at room temperature using a flow rate of 1 mL/min. The mobile phase composition was 35% sodium acetate buffer (50 mM, pH = 4.0)/65% methanol (v/v). The *O*-methylhydroxylamine product was detected with a Hitachi Model L-7480 fluorescence detector with excitation at 390 nm and emission at 490 nm. A Bruker/IBM 200D-SRC electron paramagnetic resonance spectrometer (EPR) was used occasionally to monitor the AP probe in some soil suspensions. Samples were drawn into 50 $\mu$L capillaries, which were then sealed at the top and bottom and placed within standard 3 mm i.d. quartz EPR tubes. Standard instrument settings were as follows: frequency, 9.5 GHz, microwave power, 10 mW, and modulation amplitude, 1 G. A Perkin-Elmer 2380 atomic absorption spectrophotometer (AA) was used to quantitate total iron in selected soils.

**$H_2O_2$ Measurements.** Soil suspensions were obtained by dispersing 0.6 g of soil in 15 mL of a $H_2O_2$ solution prepared with deionized water or with phosphate buffer (0.1 M, pH = 7.4) to give a 25:1 solution to soil weight ratio. Other ratios of solution to soil weight were obtained by varying the weight of soil suspended in a constant solution volume (15 mL). The slurries were agitated continuously in a rotary shaker at room temperature. At 10, 25, 40, 55, and 85 min, the total soil slurry was centrifuged for 5 min at 3000 rpm using an International Equipment Company centrifuge Model BE5G. An aliquot of the supernatant was then immediately filtered through a 0.45 $\mu$m polypropylene filter and analyzed for $H_2O_2$ concentration, providing data points every 15 min for the first hour and the last point at 1.5 h. $H_2O_2$ concentrations were determined by the dimerization of p-hydroxyphenyl acetic acid (POHPA) in the presence of horseradish peroxidase as described by Miller and Kester (*24*) with modifications (see Supporting Information).

The effects of sterilization and soil size fractions on the rates of $H_2O_2$ loss and OH formation were examined by

methods described in the Supporting Information. The iron content of selected soils was determined by AA following digestion in acid using a procedure similar to that of Bowman (*23*) and described in detail in the Supporting Information.

**OH Measurements.** EPR spectroscopy was first employed to check for the loss of the AP probe through sorption to soil particles or to the 0.2 $\mu$m nylon filter. The formation of OH was monitored in different fractions of the soil solution, including the soil supernatant, filtered and unfiltered, and the soil suspension itself (see above).

The standard reaction condition consisted of a 25:1 solution to soil weight ratio suspension, containing 100 mM DMSO, 500 $\mu$M AP, and 40 $\mu$M $H_2O_2$ in phosphate buffer (0.1 M, pH = 7.4) or in deionized water. The reaction was vigorously vortexed for 30 s and incubated in the dark at room temperature for an appropriate period. Aliquots of 500 $\mu$L were removed, centrifuged at 9000 rpm for 1 min using an Eppendorf tabletop centrifuge Model 5415 C, and filtered through a 0.2 $\mu$m nylon filter. Two hundred microliters of borate buffer (1 M, pH = 8.4) was then added to this solution and derivatized by the addition of 200 $\mu$L of the fluorescamine stock. The solution was vortexed and kept in the dark for 1 min before injecting onto the HPLC. Blanks consisted of the reaction mixture excluding either AP, DMSO, or $H_2O_2$. Excess catalase (800 units/mL) was added to the Jackland soil slurry prior to $H_2O_2$ addition as well as after the reaction proceeded for some period to examine whether the additional formation of product was eliminated. The hydroxyl radical was generated via the Fenton reaction, both in a 25:1 solution to soil weight ratio of the Berryland E soil and in the absence of the soil. The reaction mixture consisted of 500 $\mu$M AP, 100 mM DMSO, 40 $\mu$M $H_2O_2$, and 20 $\mu$M $FeSO_4$.

**Dioxygen Measurements.** Dissolved dioxygen measurements were acquired for soil suspensions using a YSI Model 58 dissolved dioxygen meter. The $O_2$ probe was sealed within a 30 mL reaction vessel to eliminate headspace. The vessel was magnetically stirred in a 25 °C water bath. The meter was calibrated by examining its response following the addition of catalase to a series of solutions containing known $H_2O_2$ concentrations. Catalase catalyzes the disproportionation of $H_2O_2$ to $O_2$, producing a concentration of $O_2$ equivalent to half of the concentration of $H_2O_2$ added. A calibration curve of detector response versus [$O_2$] was thus generated by measuring the detector response acquired for the quantity of $O_2$ evolved following addition of excess catalase (100 units/mL) to a series of solutions containing known $H_2O_2$ concentrations (0.4 $\mu$M to 100 $\mu$M).

A 25:1 solution to soil weight ratio in distilled, deionized (Milli-Q) water was used in all experiments. The soil suspension was first equilibrated for 30 min in the reaction vessel prior to $H_2O_2$ addition. An appropriate amount of $H_2O_2$ was then added through a small port on the top of the reaction vessel, with the concentration of $O_2$ and temperature then recorded every 5 min over a 90 min time period. A blank, consisting of the soil slurry in distilled, deionized water, was analyzed in the same fashion. Blanks usually showed a slow drift of 3−5 $\mu$M $O_2$ over an hour, which was subtracted from sample runs. The average of three independent runs of the sample and the blank was used to obtain [$O_2$] formed in the reaction based on the calibration curve.

## Results and Discussion

**OH Detection and Quantification.** Initial attempts to employ fluorescamine-derivatized AP (3-APF) (*15*−*17*) directly as a OH probe were unsuccessful due to its adsorption onto soil particles and the nylon filter as monitored by EPR. Using AP alone, no evidence of sorption to the soils or loss on filters was observed by EPR. Derivatization of soil supernatants with fluorescamine showed no interference from naturally occurring amines in the soils, indicating that this technique could be applied. Thus all further experiments employed AP for radical trapping, followed by derivatization with fluorescamine (*13*, *14*, *18*).

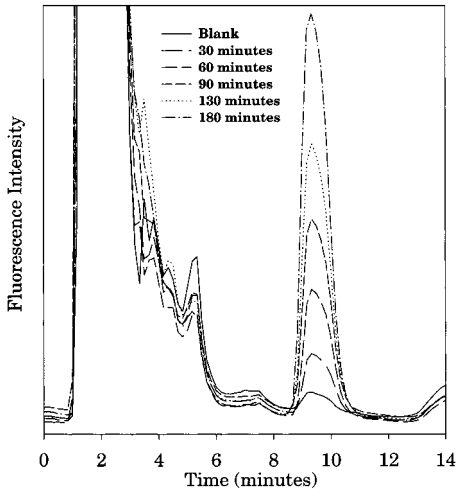


**FIGURE 1. Chromatograms illustrating the increase in *O*-methylhydroxylamine product with time in Jackland A soil slurries containing 500 $\mu$M AP, 100 mM DMSO, and 40 $\mu$M $H_2O_2$ in 0.1 M phosphate buffer at pH = 7.4. Blank denotes absence of $H_2O_2$, which is similar to that found by the absence of soil and DMSO.**

Addition of 40 $\mu$M $H_2O_2$ to a suspension of Jackland A soil containing 500 $\mu$M AP and 100 mM DMSO in phosphate buffer at pH = 7.4 produced a product eluting at ∼10 min (Figure 1). This product increased over the course of 180 min, the approximate time scale of the $H_2O_2$ decomposition. However, this product was not produced over time when $H_2O_2$, DMSO, or soil was excluded from the suspensions. A small background signal was observed in the absence of $H_2O_2$, DMSO, or the soil (Figure 1) and was subtracted from the subsequent signals. These results, combined with the coelution of this product with an *O*-methylhydroxylamine standard, indicated that this species was the *O*-methylhydroxylamine. Further, the kinetics of competition between DMSO and ethanol were consistent with OH being the detected intermediate (see Supporting Information) (*15*−*17*).

In a 25:1 solution to soil weight ratio of Berryland BHS soil containing 500 $\mu$M AP and 40 $\mu$M $H_2O_2$ in phosphate buffer at pH = 7.4, the product formation rate became independent of DMSO at concentrations above ∼50−100 mM, indicating quantitative reaction of OH with DMSO. As an additional test that OH was reacting completely with DMSO at 100 mM, we examined the product yield obtained from the Fenton reaction (20 $\mu$M $FeSO_4$, 40 $\mu$M $H_2O_2$, 500 $\mu$M AP, and 100 mM DMSO) in the presence and absence of soil. Equivalent yields of *O*-methylhydroxylamine were obtained, indicating that OH was reacting quantitatively with the DMSO, and thus that the high concentrations of organic and inorganic constituents within the soil were not competing effectively with DMSO to reduce the detected level of OH. This soil was utilized as it has a high organic matter content (2.4%), similar to the Jackland A and Myersville Ap soils (3.4−3.8%) used in later experiments.

Increasing the concentration of the AP in the 25:1 Berryland BHS soil suspension increased the rate of product formation, consistent with the known competition between the AP and dioxygen for the methyl radical. The dependence of the product formation rate on AP concentration was similar to those previously reported (*14*−*17*), providing further evidence for reaction with the methyl radical, and indicating that $O_2$ was the only significant competitive sink for this

**TABLE 2. Rate Parameters for $H_2O_2$ Decay and OH Formation in Selected Soils**

| soil type | $H_2O_2$ rate constant[a] | | OH formation rate[e] | | | |
|---|---|---|---|---|---|---|
| | $k$ ($h^{-1}$) in water | $k$ ($h^{-1}$) in PB[d] | (nM $h^{-1}$) in water | (nM $h^{-1}$) in PB[d] | organic matter,[c] % | Fe (g/kg soil)[c] |
| Berryland E 25:1[b] | ≤0.04 | 0.1 | 630<br>OH efficiency[f] (45−90 min) ∼ 30% | 735 | 0.0032 | 0.022 |
| Jackland A 25:1 | 2.1 | 2.0 | 900<br>OH efficiency<br>(0−45 min) ∼2%<br>(45−90 min) ∼35−40% | 1180 | 0.14 | 1.02 |
| Myersville Ap 25:1 | 1.6 | 1.7 | 182<br>OH efficiency<br>(0−45 min) ∼0.7%<br>(45−90 min) ∼1% | 190 | 0.15 | 1.33 |
| Christiana Bt 25:1 | 0.07 | | 52 | | 0.044 | 0.88 |
| Berryland BHS 25:1 | 0.28 | | | | 0.092 | 0.033 |
| Christiana A 25:1 | 1.2 | | | | 0.32 | 0.62 |

[a] Initial $[H_2O_2] = 40\ \mu M$ at 25 °C. [b] Solution to soil weight ratio. [c] Normalized for soil content in slurry (v/w). [d] Phosphate buffer at pH = 7.4 (0.1 M). [e] OH formation rates obtained from the slope of cumulative [OH] vs time at 25 °C. [f] Efficiency is defined as nM OH formed/nM $H_2O_2$ decomposed in PB.

radical under these aerobic conditions. Thus, product formation rates (*O*-methylhydroxylamine) were corrected to OH formation rates using the expression

$$k_f = R\left(1 + \frac{k_O[O_2]}{k_N[AP]}\right) = 2.8 \pm (0.8)\cdot R \qquad (10)$$

where R is the initial rate of product formation at 500 $\mu$M AP (*15*).

Hydroxyl radical formation rates acquired for soils at their natural acidic pH (pH = 3−5) were the same within error from those measured at pH = 7.4 in the presence of the phosphate buffer (Table 2). Like $H_2O_2$ decay (see below), the formation of OH was predominantly associated with the soil particles.

**Comparison of $H_2O_2$ Decay with OH and $O_2$ Formation.** Within the scatter of the data, $H_2O_2$ concentrations decreased in an approximately exponential fashion (Figure 2), consistent with a first-order or pseudo-first-order reaction. The rate constants were weakly correlated with the Fe and organic matter content of the soils (Table 2; see Supporting Information) and showed little or no dependence on the $H_2O_2$ concentration and, surprisingly, little dependence on pH and phosphate concentration (Table 2). At least 65−75% of the decomposition was associated with the soil particles (Supporting Information). Treatment with sodium azide and formaldehyde reduced the rate constants by 4- and 1.7-fold, respectively, whereas autoclaving produced a 2.3-fold reduction, suggesting that biological activity was at least partly responsible (Supporting Information).

A comparison of the rates of OH and $O_2$ formation with that of $H_2O_2$ decay revealed that the kinetics and magnitude of production varied substantially among soils (Figure 2). Berryland E, a soil with a low iron and organic matter content (Table 1), exhibited a very slow rate of $H_2O_2$ loss, little or no $O_2$ formation, but a relatively high rate of OH production (Figure 2A). Over the course of 90 min, the loss of $H_2O_2$ was within the measurement uncertainty, ∼3−5 $\mu$M, whereas the formation of OH was high, ∼1 $\mu$M. This result indicates that a significant fraction of the $H_2O_2$ decomposed by this soil produces OH, with efficiencies on the order of 15−30% (where efficiency is defined as nM OH formed/nM $H_2O_2$ decomposed; Table 2). For this soil, OH formation can account for much of the $H_2O_2$ lost, suggesting that the Haber-Weiss mechanism dominates the decomposition. This soil does contain small amounts of iron in the form of goethite, which may account for the significant yield of OH (*8*, *9*, *25*). In contrast, Christiana Bt, a clayey, low organic matter soil with high iron content in the form of hematite, exhibited a significantly lower rate of OH formation than the Berryland E, despite similar low rates of $H_2O_2$ loss and $O_2$ production (Figure 2B). This result suggests that iron in the form of hematite is much less effective than goethite in producing OH, consistent with past work on different forms of iron oxides (*8*, *9*, *25*). The absence of significant $O_2$ production indicates that the decomposition of $H_2O_2$ in these two soils results neither from its disproportion (via a catalase-type mechanism) nor its stoichiometric oxidation.

In contrast, for those soils exhibiting a relatively rapid loss of $H_2O_2$, the formation of $O_2$ was coupled both kinetically and stoichiometrically to the loss of $H_2O_2$ (Jackland A and Myersville Ap; Figure 2C,D). The stoichiometric ratio of $H_2O_2$ lost to $O_2$ formed was found to be ∼2 (Figure 2, reaction 8**)**, implying that disproportionation through a catalase-type mechanism dominates the loss of $H_2O_2$, particularly at early times in the decay.

Unlike $O_2$ production, the kinetics of OH formation were clearly uncoupled from $H_2O_2$ loss in these soils (Figure 2C,D). $H_2O_2$ concentrations decreased rapidly over the first 30 min, but relatively little OH was produced over this same time period. At longer times, OH continued to increase in an apparently linear fashion, whereas the $H_2O_2$ concentration asymptotically approached the detection limit of ∼80−100 nM. By ∼180 min, OH formation reached a plateau (Figure 3). Because the concentration of $H_2O_2$ at 90 min and longer was negligible within our ability to measure this species, we employed catalase to test whether sufficient $H_2O_2$ still remained in the system to drive OH formation. This enzyme efficiently catalyzes the disproportionation of $H_2O_2$ to $H_2O$ and $O_2$ (*4*, *15*), thus removing $H_2O_2$ from the suspension. Introduction of catalase after allowing the reaction to proceed for 75 min substantially reduced the formation of additional OH (Figure 3). Further, the addition of catalase to this soil suspension prior to the addition of $H_2O_2$ eliminated OH

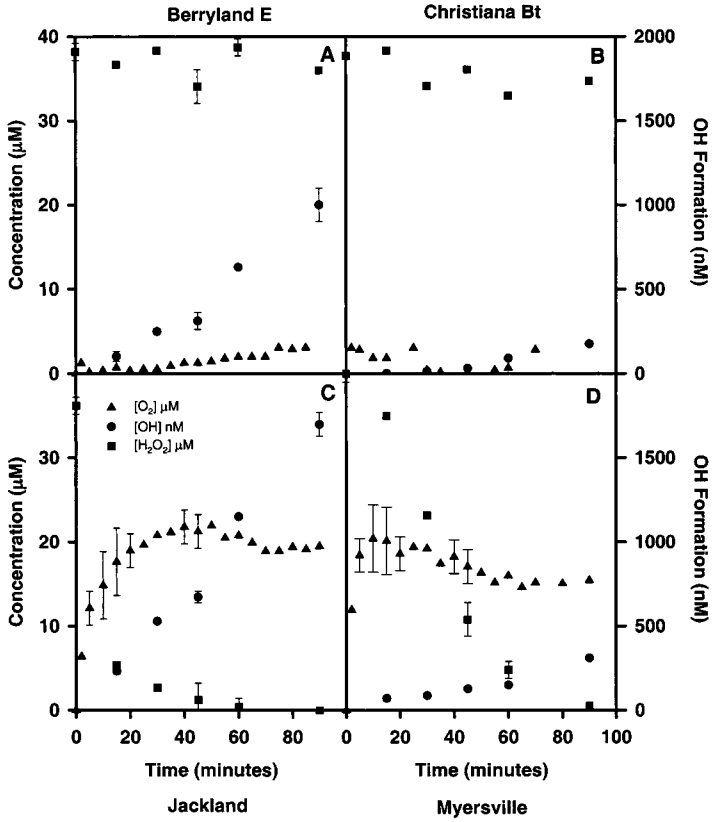


**FIGURE 2. Time courses for $H_2O_2$ loss and $O_2$ and OH formation: initial [$H_2O_2$] = 40 $\mu$M, A = Berryland E, B = Christiana Bt, C = Jackland A, and D = Myersville Ap (25:1 solution-to-soil ratio) in 0.1 M phosphate buffer, pH = 7.4. Error bars represent the standard deviation for three replicate samples.**

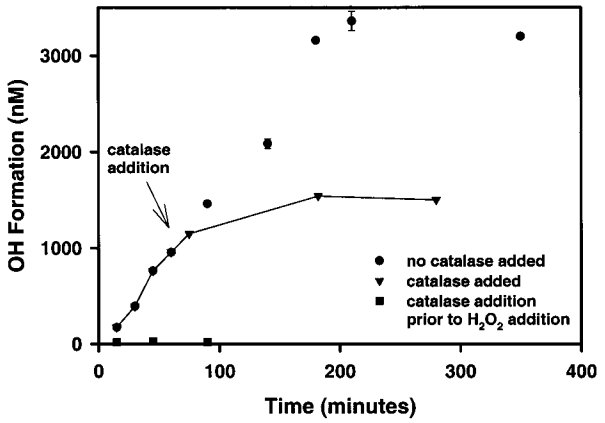


**FIGURE 3. Effect of catalase on the time course of OH formation in Jackland A soil slurries (25:1 solution to soil weight ratio) containing 500 $\mu$M AP, 100 mM DMSO, and 40 $\mu$M $H_2O_2$ in 0.1 M phosphate buffer at pH = 7.4. Catalase (800 units/mL) was added prior to $H_2O_2$ addition as well as after the reaction proceeded for 75 min. Error bars represent the standard deviation for three replicate samples.**

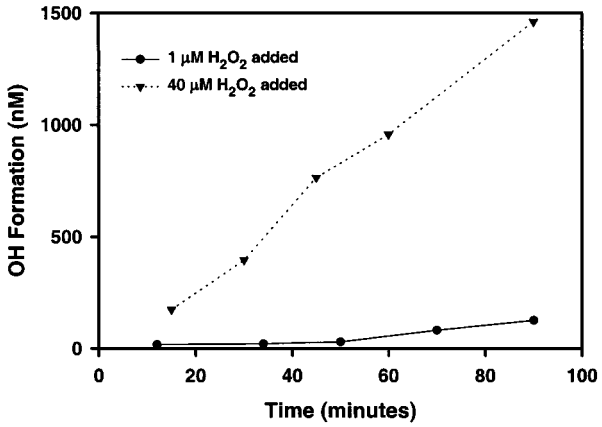


**FIGURE 4. Dependence of the time course for OH formation on $H_2O_2$ concentration in Jackland A soil slurries (25:1 solution to soil weight ratios) containing 500 $\mu$M AP and 100 mM DMSO in 0.1 M phosphate buffer at pH = 7.4.**

formation completely. These results indicate that residual $H_2O_2$ in the solution was the source of the OH and that the presence of $H_2O_2$ was necessary for the formation of OH.

Because $H_2O_2$ concentrations decreased in an approximately exponential fashion, but OH formation increased linearly, the efficiency of OH formation appeared to increase as the $H_2O_2$ concentration decreased. For example, in the Jackland A soil, about 37 $\mu$M $H_2O_2$ was consumed and 700 nM OH produced over the first 45 min, leading to an efficiency of OH production of ~2%. However, from 45 to 90 min, ~2 $\mu$M of $H_2O_2$ was consumed and ~700−800 nM OH was produced leading to a OH production efficiency of ~35−40%. At later times, this efficiency appears to have risen even higher. Although the efficiency of OH production increased substantially with decreasing $H_2O_2$ concentration, the overall efficiency of OH production for a single 40 $\mu$M addition of $H_2O_2$ was only ~8% for the Jackland A soil following complete decomposition of $H_2O_2$ after 180 min (Figure 2C).

To test directly whether the efficiency of OH formation increased with decreasing $H_2O_2$, OH formation was followed at high and low concentrations of $H_2O_2$ in the Jackland A soil over 90 min (Figure 4). Decreasing the initial $H_2O_2$ concentration by 40-fold, from 40 to 1 $\mu$M, decreased the rate of OH production by only ~10-fold. The efficiency of OH formation increased from ~4 to ~10% for the decomposition of 40 $\mu$M

and 1 $\mu$M $H_2O_2$, respectively (Figure 4). By 90 min after the addition of 1 $\mu$M $H_2O_2$, 100 nM of OH was already formed, representing 10% of the total $H_2O_2$ added. This value represents a lower bound on the overall efficiency of OH formation at this concentration of $H_2O_2$ and is still higher than the overall yield of 8% for the Jackland A soil upon complete decomposition of 40 $\mu$M $H_2O_2$ at 180 min (Figure 3). These results also indicate that OH is produced at higher efficiencies at lower concentrations of $H_2O_2$ (Figure 4).

This type of kinetic behavior could be explained by the presence of two classes of catalytic sites: one (catalase-type) with a relatively low affinity for $H_2O_2$ but with a high turnover rate and one (Haber-Weiss) with a high affinity for $H_2O_2$ but a much lower turnover rate. At higher concentrations of $H_2O_2$, the low affinity site would remain undersaturated with respect to $H_2O_2$ (giving rise to first-order decay kinetics) but would still dominate the total decay due to the higher catalytic rate. The high affinity, OH producing site would remain saturated with $H_2O_2$ (giving rise to zero-order decay kinetics) but would dominate the decay only at later times when the $H_2O_2$ concentration was depleted. Although this explanation is compatible with the data presented in Figures 2 and 3, it is inconsistent with the data presented in Figure 4, which indicates that OH formation rates are dependent on $H_2O_2$ concentration. Clearly, a more complete understanding of the reactions occurring within these complex soil systems will require additional kinetic studies. Regardless, these combined results indicate the presence of two distinct pathways for $H_2O_2$ decomposition in the Jackland A and Myersville Ap soils (Figure 2C,D): one decomposition pathway (the catalase-type) that dominates at earlier times and a second, OH-producing pathway that appears to dominate at later times when $H_2O_2$ concentrations are depleted.

Our observation that $H_2O_2$ decay and OH formation are primarily localized on soil particles is consistent with the idea that these reactions are occurring via particle-associated microorganisms or enzymes and/or on surface (oxide) sites (*4*, *8*−*10*, *25*). The decrease in the $H_2O_2$ decay rates after sterilization further supports the idea that biological activity is involved.

Because the loss of $H_2O_2$ can largely be explained by the production of $O_2$ and OH within these soils (with the possible exception of the Christiana Bt soil, Figure 2), our results suggest that disproportionation through a catalase-type mechanism and the production of OH through a Haber-Weiss mechanism represent the principal routes through which $H_2O_2$ is lost. These results stand in contrast to those reported for aquatic systems where 30−70% of the peroxide decay could be assigned to peroxidase activity (*26*, *27*). Kerstetter et al. (*28*) found evidence for peroxidase activity in surface soils but did not quantify the fraction of the total $H_2O_2$ decomposed that could be attributed to this activity. Our data imply that the oxidation by $H_2O_2$ of organic soil constituents, whether natural or anthropogenic, will occur primarily via reaction with OH and not through peroxidic reactions. Although the precise factors controlling the relative magnitudes of the catalase-type and Haber-Weiss pathways have yet to be revealed, our data suggest that in soils containing higher levels of organic matter (or perhaps Mn), the disproportionation pathway will dominate, whereas in soils containing lower levels of some metals, possibly iron in the form of goethite, the Haber-Weiss pathway will dominate.

Finally, we have applied a new approach (*13*−*18*) that unlike previous methods (*6*) can provide highly sensitive, quantitative estimates of OH production in complex soil suspensions. A comparison between the rates of $H_2O_2$ loss and OH formation indicates that the yield of OH can vary widely, apparently depending on numerous soil parameters as evidenced by the lack of a clear correlation with any single bulk parameter such as organic matter or iron content. Nevertheless, we have observed that those soils exhibiting higher rates of $H_2O_2$ loss generally have lower yields of OH due to the presence of a more rapid, competing $H_2O_2$ decomposition process (catalase-type). Although the rates of OH formation in these soils can be significant, the faster competing process ultimately limits the overall yield of OH. In contrast, for those soils exhibiting slow rates of $H_2O_2$ decay, the yield of OH can be quite high, although the absolute rates of OH production are no larger, and in some cases smaller, than those observed in soils with rapid loss of $H_2O_2$ (Figure 2). This new technique thus offers the possibility of determining the importance of OH production in both natural and remediated soils.

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## Supporting Information Available

Experimental details of the analyses of $H_2O_2$ and soil iron content, the sterilization and size fractionation of soils, and the kinetics of $H_2O_2$ loss as well as additional tests of the method for OH determination are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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